

U.S. Patent Application Serial No. 09/340,196
Response filed September 3, 2004
Reply to OA dated April 8, 2004

IN THE CLAIMS

Please amend claims 76 and 77, as follows:

Claims 1-50 (Cancelled).

Claim 51 (Previously presented): A method for determining malignancy of a thyroid tumor, the steps comprising:

- (a)(i) adding to a fluid sample originating from a living body, a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin; and
- (ii) adding to the sample an anti-thyroglobulin antibody capable of binding to both the first type of thyroglobulin and the second type of thyroglobulin; and
- (b)(i) measuring an amount of conjugates formed in step (a)(i) of said specific lectin or said specific antibody with the first type of thyroglobulin; and
- (ii) measuring a total amount of conjugates formed in step (a)(ii) of the anti-thyroglobulin antibody with the first and second types of thyroglobulin; and
- (c) determining the malignancy of a thyroid tumor by comparing the calculated ratio of the amounts measured in (b)(i) and (b)(ii) with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

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wherein the sample is determined to be malignant when the calculated ratio is significantly higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claim 52 (Canceled).

Claim 53 (Previously presented): A method for determining malignancy of a thyroid tumor from a fluid sample originating from a living body, the steps comprising:

(1) measuring an amount of one of two types of thyroglobulin in a fluid sample originating from a living body, the steps comprising:

- (a) providing a reagent comprising,
 - (i) an anti-thyroglobulin antibody capable of binding to both a first type of thyroglobulin and a second type of thyroglobulin,
 - (ii) a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of the first type of thyroglobulin but not capable of binding to the sugar chain of the second type of thyroglobulin;

(b) adding to the fluid sample said reagent; and

(c)(i) measuring a total amount of conjugate formed in step (b) of the anti-thyroglobulin antibody with both of the first and second types of thyroglobulin; and

- (ii) measuring an amount of conjugate formed in step (b) of said specific lectin or said

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specific antibody with the first type of thyroglobulin,

(2) determining the malignancy of the thyroid tumor by comparing a calculated ratio of the amount measured in (c)(ii) to the amount of total thyroglobulin measured in (c)(i) in the sample with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant when the calculated ratio is significantly higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claim 54 (Previously presented): The method of claim 53, wherein the method further comprises separating a conjugate formed in step (b) prior to measuring the amount of the conjugate.

Claim 55 (Canceled).

Claim 56 (Previously presented): A method of determining malignancy of a thyroid tumor comprising:

(1) measuring a total amount of a thyroglobulin in a fluid sample originating from a living body and (a) an amount of a first type of thyroglobulin having a first sugar chain with a specific structure to which a specific lectin or a specific antibody is capable of binding; or, (b) an amount of

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a second type of thyroglobulin having a sugar chain to which the specific lectin or specific antibody is not capable of binding;

(2) calculating a ratio of (a) the amount of the first type of thyroglobulin to the total thyroglobulin; or (b) the amount of the second type of thyroglobulin to the total thyroglobulin; and
(3) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant when the calculated ratio is significantly higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claims 57 - 58 (Canceled).

Claim 59 (Previously presented): A method for determining malignancy of a thyroid tumor comprising:

(1) measuring an amount of one of two types of thyroglobulin in a fluid sample originating from a living body, the steps comprising:
(a) adding to the sample a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a

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sugar chain of a second type of thyroglobulin, to form a conjugate of the specific lectin or the specific antibody with the first type of thyroglobulin;

- (b) separating said conjugate from the non-conjugated second type of thyroglobulin;
 - (c) measuring said conjugate content, for determining the amount of the first type of thyroglobulin; or
 - (d) measuring an amount of the non-conjugated second type of thyroglobulin,
- (2) determining malignancy of the thyroid tumor by comparing a calculated ratio of the amount measured in (c) or (d) to an amount of total thyroglobulin in the sample with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;
wherein the sample is determined to be malignant when the calculated ratio is significantly higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claims 60 - 67 (Canceled).

Claim 68 (Previously presented): A method for determining malignancy of a thyroid tumor comprising the steps of:

- (a) adding to a fluid sample originating from a living body:
 - (i) a specific lectin or a specific antibody capable of binding to a specific

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structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, and

(ii) a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin, to form a first conjugate which is a conjugate of the first anti-thyroglobulin antibody with the first type of thyroglobulin and with the specific lectin or the specific antibody, and a second conjugate which is a conjugate of the first anti-thyroglobulin antibody with the second type of thyroglobulin;

(b) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and

(c) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;

(d) calculating a ratio of (a) the amount of the first type of thyroglobulin to the amount of total thyroglobulin; or (b) the amount of second type of thyroglobulin to the amount of total thyroglobulin; and

(e) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant when the calculated ratio is significantly

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higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claim 69 (Previously presented): A method for determining malignancy of a thyroid tumor comprising the steps of:

- (a) adding to a fluid sample originating from a living body, a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,
 - to form a conjugate of the specific lectin or the specific antibody with the first type of thyroglobulin;
- (b) separating the conjugate from the second type of thyroglobulin; and
- (c) measuring an amount of the first type of thyroglobulin on the basis of the conjugate content; and
- (d) measuring an amount of the separated second type of thyroglobulin;
- (e) calculating a ratio of (a) the amount of the first type of thyroglobulin to the amount of total thyroglobulin; or (b) the amount of second type of thyroglobulin to the amount of total thyroglobulin; and
- (f) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a

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benign thyroid;

wherein the sample is determined to be malignant when the calculated ratio is significantly higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claim 70 (Previously presented): A method for determining malignancy of a thyroid tumor comprising the steps of:

- (a) adding to a fluid sample originating from a living body:
 - (i) a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,
 - (ii) a first anti-thyroglobulin antibody capable of binding to both the first type of thyroglobulin and the second type of thyroglobulin, and
 - (iii) a second anti-thyroglobulin antibody capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin or the specific antibody is already bound,

to form a first conjugate which is a conjugate of the first anti-thyroglobulin antibody with the first type of thyroglobulin and with the specific lectin or the specific antibody, and a second conjugate which is a conjugate of the first anti-thyroglobulin antibody with the second type of thyroglobulin and the second anti-thyroglobulin antibody;

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- (b) separating the first conjugate and the second conjugate; and
- (c) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and
- (d) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;
- (e) calculating a ratio of (a) the amount of the first type of thyroglobulin to the amount of total thyroglobulin; or (b) the amount of second type of thyroglobulin to the amount of total thyroglobulin; and
- (f) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;
wherein the sample is determined to be malignant when the calculated ratio is significantly higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claim 71 (Previously presented): A method for determining malignancy of a thyroid tumor comprising the steps of:

- (a) adding to a sample originating from a living body:
 - (i) a specific lectin or a specific antibody capable of binding to a specific

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structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, and

- (ii) an anti-thyroglobulin antibody-2 capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin or the specific antibody is already bound,

to form a first conjugate which is a conjugate of the specific lectin or the specific antibody with the first type of thyroglobulin, and a second conjugate which is a conjugate of the anti-thyroglobulin antibody-2 with the second type of thyroglobulin;

- (b) separating the first conjugate and the second conjugate formed in the step (a);
- (c) adding an anti-thyroglobulin antibody-1 capable of binding to both types of thyroglobulin to the second conjugate formed in the step (a), to form a third conjugate which is a conjugate of the anti-thyroglobulin antibody-2 with the second type of thyroglobulin and with the anti-thyroglobulin antibody-1;
- (d) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content and
- (e) measuring an amount of the second type of thyroglobulin on the basis of the third conjugate content;
- (f) calculating a ratio of (a) the amount of the first type of thyroglobulin to the amount of total thyroglobulin; or (b) the amount of second type of thyroglobulin to the amount of total thyroglobulin; and

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(g) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant when the calculated ratio is significantly higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claim 72 (Previously presented): A method for determining malignancy of a thyroid tumor comprising the steps of:

(a) adding to a sample originating from a living body:

- (i) a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, and
- (ii) an anti-thyroglobulin antibody-2 capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin or the specific antibody is already bound,

to form a first conjugate which is a conjugate of the specific lectin or the specific antibody with the first type of thyroglobulin, and a second conjugate which is a conjugate of the anti-thyroglobulin antibody-2 with the second type of thyroglobulin;

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- (b) separating the first conjugate and the second conjugate; and
- (c) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and
- (d) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;
- (e) calculating a ratio of (a) the amount of the first type of thyroglobulin to the amount of total thyroglobulin; or (b) the amount of second type of thyroglobulin to the amount of total thyroglobulin; and
- (f) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;
wherein the sample is determined to be malignant when the calculated ratio is significantly higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claim 73 (Previously presented): A method for determining malignancy of a thyroid tumor comprising the steps of:

- (a) dividing a fluid sample originating from a living body into a first portion and a second portion;

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(b)(i) adding to the first portion a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,

to permit the precipitation of a conjugate of the first type of thyroglobulin with the specific lectin, or the first type of thyroglobulin with the specific antibody;

(ii) separating the precipitated conjugate from the second type of thyroglobulin; and

(iii) measuring an amount of the second type of thyroglobulin of the separated part first portion; and

(c)(i) measuring an amount of the total thyroglobulin of the second portion; and

(ii) determining an amount of the first type of thyroglobulin from the difference between an amount of the total thyroglobulin and the amount of the second type of thyroglobulin obtained in step (b)(iii);

(d) calculating a ratio of (a) the amount of the first type of thyroglobulin to the amount of total thyroglobulin; or (b) the amount of second type of thyroglobulin to the amount of total thyroglobulin; and

(e)determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant when the calculated ratio is significantly

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higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claim 74 (Previously presented): A method for determining malignancy of a thyroid tumor comprising the steps of:

- (a) adding to a fluid sample originating from a living body, a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin; then
- (b) adding to the sample a first antibody, capable of binding to both types of thyroglobulin, to form a first conjugate which is a conjugate of the first antibody with the first type of thyroglobulin and with the specific lectin or the specific antibody, and a second conjugate which is a conjugate of the first antibody with the second type of thyroglobulin;
- (c) separating the first conjugate and the second conjugate; and
- (d) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and
- (e) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;
- (f) calculating a ratio of (a) the amount of the first type of thyroglobulin to the amount of total thyroglobulin; or (b) the amount of second type of thyroglobulin to the amount of total thyroglobulin; and

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(g) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant when the calculated ratio is significantly higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claim 75 (Previously presented): A method for determining malignancy of a thyroid tumor comprising:

(a) dividing a fluid originating from a living body into a first portion and a second portion;

(b)(i) adding to the first portion a specific lectin or a specific antibody capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,

to form a conjugate of the first type of thyroglobulin with the specific lectin or the specific antibody; then

(ii) adding to the first portion an antibody-2 capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin or the specific antibody is already bound, to form a conjugate of the second

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type of thyroglobulin with the antibody-2; and

(iii) measuring the amount of the second type of thyroglobulin on the basis of the measurement of the second type of thyroglobulin with antibody-2 conjugate formed in step (b)(ii); and

(c)(i) measuring an amount of the total thyroglobulin of the second portion; and

(ii) determining an amount of the first type of thyroglobulin from the difference between an amount of the total thyroglobulin and the amount of the second type of thyroglobulin obtained in step (b)(iii);

(d) calculating a ratio of (a) the amount of the first type of thyroglobulin to the amount of total thyroglobulin; or (b) the amount of second type of thyroglobulin to the amount of total thyroglobulin; and

(e) determining the malignancy of a thyroid tumor by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant when the calculated ratio is significantly higher or lower than that of the reference fluid sample of the normal thyroid and is significantly higher or lower than that of the reference fluid sample of the benign thyroid.

Claim 76 (Currently amended): The method of any one of claims 51, 53, 56, 59 and 68-75,

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wherein said specific antibody is one reactive with an Lewis type sugar chain.

Claim 77 (Currently amended): The method according to any one of claims 51, 53, 56, 59 and 68-75, wherein the sugar chain with the specific structure is one found in thyroglobulin which is produced by a carcinoma cell.